

### Abstract of the Disclosure

A method of biological assay comprises the steps of providing an enzyme substrate comprising two fluorescence dye groups bound to a flexible peptide, the dye groups being of proximity sufficiently close so as to allow free energy  
5 attractions to draw the dyes together to essentially self-quench fluorescence of the dye groups, wherein self quenching of fluorescence of the dye groups is effected by dye dimerization or stacking, and enzymatically cleaving the peptide to release the fluorescence dye groups from dye dimerization or stacking, thereby producing an increase in fluorescence intensity. A protease substrate for use in the method of the  
10 invention is also disclosed. This invention finds use in detection and identification of microorganisms, sterilization assurance, pharmaceutical discovery, enzyme assays, immunoassays, and other biological assays.

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